REMARKS

Objection to the claims:

Claims 1 and 25 have been amended as recommended by the examiner to obviate the objections.

Rejection of the Claims under 35 USC § 112:

Claims 1 and 25 have been amended to obviate the rejection. Specifically the term "determinant of said antigen" has been amended to "antigenic determinant of said antigen."

Claim 8 has been canceled.

Rejection of the claims under 35 USC § 102:

Claims 1-6, 9-12, 25 and 27 have been rejected under 35 U.S.C. 1-2(b) as being anticipated by either one of Liu et al (1999) or Zhang et al. (1999). As discussed in the Nov. 18 telephone interview, Applicants have amended the claims to clarify their new inventive use. 35 U.S.C. 100 has been found to allow for a patent for a process, including a new use of a known process (MPEP 706.03(a)). The discovery of a new use for an old structure based on unknown properties of the structure is patentable to the discoverer as a process of using. (MPEP 2112.02). MPEP 806.05(h) further states that a product and a process of using that product can be shown to be distinct inventions.

Liu et al. and Zhang et al. disclose a method for delivering plasmid DNA to a liver cell via injection into tail vein and expressing the plasmid DNA in the liver cell. Liu et al. list several potential uses for their technique on page 1265, first column, but do not mention genetic immunization, genetic vaccination, or generation of an immune response. Similarly, Zhang et al. list potential uses for their technique without mention of generation of an immune response (page 1737, first column, last paragraph). There is no evidence in the publications of Liu et al. or Zhang et al. to indicate that it was known to them at the time of publication that it was possible to generate an immune response to an expressed antigen following injection of DNA into the tail vein of a mouse. Therefore, neither Liu or Zhang were in possession of every limitation of the applicants' claim. That the invention was not "at once apparent to any one skilled in the art" is evidenced by Crispe (Nature Reviews Immunology 2003 Vol. 3 pp. 51-62), Lau et al. (Gut 2003 Vol. 52, pp. 307-314), and Knolle et al. (Immunol Rev. 2000

Vol. 174, pp. 21-34). The method of Liu and Zhang delivered plasmid DNA primarily to the liver. Crispe, Lau et al. and Knolle et al. all describe the significantly different immune properties of the liver compared with other tissues. More specifically, they each describe the suppressed immune responsiveness of the liver.

The action states on page 8 that "The specification teaches that the antibody response obtained by this method, 'is not surprising given the large amount of antigen that is produced.' (page 41, line 20-21" Applicants respectfully note that the example in which this statement is made initially describes intravascular delivery of naked plasmid DNA or plasmid DNA complexed with linear polyethylenimine (lPEI) and polyacrylamide (PAA). Intravascular delivery of the naked DNA or DNA complexed with lPEI and PAA was shown to result generation of an immune response to the expressed antigen. The statement in question is then related to the observation that antibody generation is increased in mice in which the DNA is delivered with TransIT *In Vivo*. Therefore, the statement does not constitute an obvious expectation of the initial result – that intravascular delivery of DNA will result in generation of an immune response – but rather is made with foreknowledge of the initial result.

Support for the amendments can be found in the specification as follows: vaccinating the mammal (page 6 lines 16-30 and page 32 lines 1-19); immunizing the mammal (page 18 lines 15-26); inducing a cellular or humoral immune response (page 4 lines 5-20, page 19 lines 13-17, and page 30 line 34 to page 31 line 7); generating antibodies or antibody producing cells (page 10 line 21 to page 11 line 25, page 20 lines 18-31, and page 36 lines 17-34).

Rejection of the claims under 35 USC § 103:

Claims 1, 7 and 26 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Liu et al. and Zhang et al. in view of Smyth-Templeton et al. It is the Applicants' opinion, as detailed above in response to the 35 U.S.C. 102 rejections, that Liu et al. and Zhang et al. did not disclose a method for genetic immunization. Therefore, it is the Applicants' opinion that it could not have been obvious to combine the teaches of either Liu et al. or Zhang et al. with the teaching of Smyth-Templeton et al.

The Examiner's objections and rejections are now believed to be overcome by this response to the Office Action. In view of Applicants' amendment and arguments, it is submitted that claims 1-7, 9-12 and 25-27 should be allowable. Applicants respectfully request a timely Notice of Allowance be issued in the case.

Respectfully submitted,

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I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as express mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this date: 12/03/2004.

Kirk Ekena

HEPATIC T CELLS AND LIVER TOLERANCE

Ian Nicholas Crispe

The T-cell biology of the liver is unlike that of any other organ. The local lymphocyte population is enriched in natural killer (NK) and NKT cells, which might have crucial roles in the recruitment of circulating T cells. A large macrophage population and the efficient trafficking of dendritic cells from sinusoidal blood to lymph promote antigen trapping and T-cell priming, but the local presentation of antigen causes T-cell inactivation, tolerance and apoptosis. These local mechanisms might result from the need to maintain immunological silence to harmless antigenic material in food. The overall bias of intrahepatic T-cell responses towards tolerance might account for the survival of liver allografts and for the persistence of some liver pathogens.

SINUSOID
A blood-filled space that lacks the anatomy of a capillary. Sinusoids generally contain slow-flowing blood, which facilitates cellular interactions. Such vessels are found in the bone marrow and in the liver.

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The liver is an organ in which blood from the intestines, which is rich in bacterial products and in mainly harmless food-derived antigens, interacts with the circulating T-cell pool. The constitutive presence of non-self and microbial molecules imposes constraints on immune responses that are generated in the liver, and there might be distinctive control mechanisms that determine whether antigen encounter will result in immunity or tolerance.

Reasons to explain why the liver is often a site of immune tolerance have not been established, but one reasonable speculation is that it is because the liver is a site where harmless food antigens from the gut are processed and presented to the immune system. However, the liver is also subjected to invasion by pathogens that breach the intestinal mucosa and invade the circulation. Immune tolerance towards such invaders would not be advantageous, which indicates that liver lymphocytes must be able to switch rapidly from a tolerant to a responsive state.

Liver dendritic cells (DCs) facilitate the priming of T cells, and virus infections of the liver can generate T-cell immunity that allows the infection to be cleared, as is generally the case for hepatitis A virus in healthy humans and mouse hepatitis virus in immunocompetent mice[1–3]. However, several infections of the liver persist despite the development of an immune response, including three that are of great epidemiological significance: malaria, hepatitis B virus (HBV) and hepatitis C virus (HCV)[4–6] (BOX 1). Also, early in the history of experimental transplantation, immunologists were surprised to discover that in many species, allogeneic liver grafts can be established and maintained without immunosuppression[7]. By contrast, skin, kidney and other allografts are rejected rapidly. These results raise the question of whether there is a global phenomenon of 'liver tolerance'. In support of this concept, tumours that often metastasize to the liver include malignant melanoma, which expresses well-defined tumour antigens[8], as well as breast and lung cancers in humans. This indicates that the liver might be a site in which immunogenic tumour cells can evade immune surveillance.

To attempt to understand the control of tolerance and immunity in the liver, I discuss the anatomy of the liver blood vessels (SINUSOIDS), which facilitate immune-cell interactions. The large macrophage population of the liver is described briefly, as are the unusual lymphocyte populations that are present in this organ. However, these considerations do not provide an easy explanation for the baseline state of T-cell tolerance, because the lymphocyte populations that are unusually abundant in the liver have a well-defined role in promoting anti-pathogen immunity. The 'professional' antigen-presenting cells (APCs) of the liver include DCs and an unusual type of vascular endothelial cell,

POLYPROTEIN
A large protein that must be cleaved to yield many functional proteins. The ten proteins of hepatitis C virus are synthesized as a single polyprotein.

LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSECs). These cells form the lining endothelium of the hepatic sinusoids. They have unusual morphology (with many small holes and no basement membrane) and unusual properties as antigen-presenting cells (a strong predisposition towards the induction of tolerance, despite the expression of many co-stimulatory molecules).

SIEVE PLATE
A cluster of small holes (fenestrae) in a liver sinusoidal endothelial cell, which is believed to facilitate diffusion between the hepatic sinusoid and the underlying space of Disse, which is where solutes can interact with hepatocytes.

KUPFFER CELLS
The macrophages of the liver. These cells are derived from blood monocytes, and they phagocytose particles, including bacteria, that enter the liver sinusoids.

LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSECs). Among these cell populations, evidence has been found for antigen-presenting function that results in T-cell inactivation, both through the induction of apoptosis and through other mechanisms.

Anatomy, shaped by function

The liver stands between the gastrointestinal tract and the systemic circulation. Blood from the gut, which is rich in food antigens, environmental toxins and bacterial products, including endotoxin (lipopolysaccharide, LPS), is collected in the hepatic portal vein (FIG. 1). This vessel is the main source of blood flowing through the liver, but approximately 20% of the incoming blood arrives in the hepatic artery. These two blood supplies mix in the hepatic sinusoids, where the blood percolates from the portal tracts to the central veins, passing between plates of hepatocytes through spaces that are lined by LSECs (FIG. 2a). This organization maximizes the exchange of molecules between the sinusoidal space and hepatocytes, allowing the liver to carry out its functions of digestion, detoxification and synthesis of plasma proteins. The sinusoidal endothelium is fenestrated, and the presence of clusters of small holes (known as SIEVE PLATES) raises the possibility that cells in the sinusoidal space might make direct contact with underlying connective tissue and hepatocytes[9]. Blood plasma, lymphocytes and DC precursors pass from the sinusoids into a sub-endothelial space, known as the space of Disse. From this space, lymph is collected, and it flows through lymphatic vessels that run in the portal tracts to the draining lymph nodes (FIG. 2b). The combination of slow blood flow, fenestrated endothelium and lack of a discrete basement membrane distinguishes liver sinusoids from other vascular beds, and it might provide T cells that pass through the liver with unique access to tissue cells.

Kupffer cells

The liver contains a large population of resident macrophages, known as KUPFFER CELLS. These cells are derived from blood monocytes, and they are found mainly in the hepatic sinusoids. However, Kupffer cells can sometimes pass through the space of Disse and make direct contact with hepatocytes[10]. They are mobile and actively phagocytic, and they can be marked and observed *in vivo* through their capacity to endocytose fluorescent microspheres[11]. Similar to other macrophages, Kupffer cells can phagocytose apoptotic cells[12,13] and microorganisms[14–16]. The interactions between Kupffer cells and T cells have been analysed *in vitro* using purified Kupffer cells, and *in vivo* by depletion techniques. In some studies, Kupffer cells from various species have been shown to act as effective APCs, resulting in T-cell proliferation and cytokine synthesis[17,18]. However, Kupffer cells might be involved also in tolerance. *In vitro*, the synthesis of nitric oxide by Kupffer cells causes them to suppress T-cell activation[19]. *In vivo*, systemic immune tolerance occurs in response to alloantigenic leukocytes injected into the portal vein[20]; this form of tolerance depends on Kupffer cells, because it is impaired if the Kupffer cells are depleted by treatment with gadolinium chloride[21].

Intrahepatic lymphocytes

The liver contains an unusual population of resident lymphocytes, among which $CD8^+$ T cells usually outnumber $CD4^+$ T cells, and both natural killer (NK) and natural killer T (NKT) cells are enriched relative to their proportions in lymphoid tissues[22,23]. Most of the intrahepatic $CD8^+$ and $CD4^+$ T cells have an activated phenotype. So, human liver $CD8^+$ T cells express CD25 and CD69 (REF. 24). In mice, the liver can trap activated $CD8^+$ T cells preferentially in perfusion experiments[25]. The intrahepatic $CD4^+$ T cells of mice have a $CD45RB^{low}$ phenotype, and they synthesize both interferon-γ (IFN-γ) and interleukin-4 (IL-4)[26]. When such activated $CD4^+$ T cells are delivered by adoptive transfer, some survive in the liver for weeks. Among these cells, those with a T helper 1 (T_H1)-type effector function become non-functional, whereas T_H2-biased $CD4^+$ T cells sustain their function[27]. In addition, a subset of liver T cells express the CD45 isoform B220, which is expressed more commonly by B cells[28]. The expression of B220 by T cells is associated with apoptosis, both *in vitro*[29] and in the liver[30].

The NK cells of rodent livers were identified first as 'pit cells', which were defined as large granular lymphocytes with cytotoxic activity against classic NK-cell targets, such as YAC1 cells[31]. These NK cells have long been known to increase in number during infection of the liver[32]. They express germline-encoded activating and inhibitory receptors, and they are present at an unusually high frequency among resident liver lymphocytes. During experimental liver injury induced by the injection of

Box 1 | Immune evasion by hepatitis C virus

Hepatitis C virus (HCV) is a main cause of liver disease in humans. HCV is a single-stranded RNA virus, for which a 9.5-kb messenger RNA encodes a single POLYPROTEIN. This polyprotein is cleaved by a combination of cellular and HCV-encoded peptidases into ten fragments. The World Health Organisation estimates that 170 million people are infected with HCV worldwide, and almost 4 million individuals are infected in the United States[150].

HCV primes the T-cell system, and $CD4^+$ and $CD8^+$ T-cell responses can sometimes co-exist with persistent infection[151,152]. In both chimpanzees and humans, an early and diverse $CD4^+$ and $CD8^+$ T-cell response is associated with clearance of the virus[153,154]. Sequence analysis of HCV isolates shows that HCV generates escape mutations in both of the two susceptible species[155–157]. This is a classic type of immune evasion, which is also seen for other persistent viruses.

HCV might disable T cells by other mechanisms also. Some mutations create antagonistic peptides with the potential to inactivate T cells that are specific for the prototype HCV antigenic epitope[157,158]. Such antagonism might explain the long-term persistence of individual virus genotypes, even in the presence of T-cell priming. In addition, HCV-specific T cells might become dysfunctional, particularly with regard to the synthesis of inflammatory cytokines. This has been termed 'stunning'[159]. The mechanism is not known.

Two HCV structural proteins, core protein and E2, have immunosuppressive properties *in vitro*. HCV core protein suppresses T-cell activation by binding to the C1q receptor[160,161], and it might also promote CD95 (FAS)-induced apoptosis[162]. The HCV envelope protein E2 binds CD81 on natural killer (NK) cells and inhibits NK-cell function[163]. The interpretation of this finding is complicated, because transgenic mice that express HCV core, E1 and E2 proteins have no evidence of global immunosuppression[164].

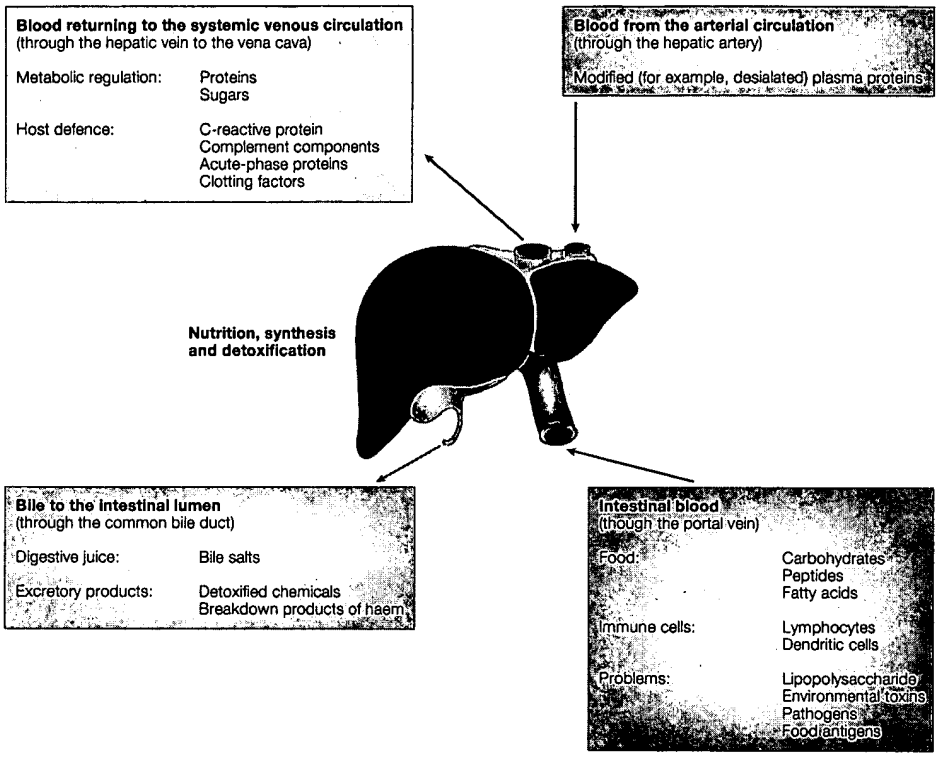

Figure 1 | **The inputs, outputs and main functions of the liver.** The figure shows that, in addition to its central metabolic role, and its interactions with T cells, which are summarized in this review, the liver makes an important contribution to host defence by synthesizing several defensive molecules, including complement components and clotting factors.

concanavalin A[33] and during infection of the liver with adenovirus vectors[34] (BOX 2), NK cells seem to have a crucial role in T-cell recruitment. During cytomegalovirus infection of mice, type 1 IFNs (IFN-α/β) induce synthesis of the chemokine CCL3 (also known as monocyte inflammatory protein-1α, MIP1α), which, in turn, is responsible for NK-cell accumulation[35]. Lack of CCL3 compromises both NK-cell accumulation and protective immunity. The NK cells synthesize IFN-γ, which promotes secretion of the chemokine CXCL9 (monokine induced by γ-interferon, Mig), probably by hepatocytes and/or LSECs[36], and this is responsible for the accumulation of T cells. Therefore, liver NK cells recruit T cells through a multi-step cytokine/chemokine cascade (FIG. 3), and in so doing, they promote T-cell immunity, rather than tolerance.

The normal mouse liver is also rich in NKT cells. These cells have a complex phenotype, features of which include the expression of NK1.1 in the B6 mouse and expression of the p70 chain of the IL-2 receptor (IL-2Rβ)[37]. The prototype NKT cells express, at intermediate density, a T-cell receptor (TCR)αβ with specificity for the MHC class-I-like molecule CD1d. Their TCR repertoire has limited diversity; the TCR β-chain is limited to Vβ8.2, Vβ7 or Vβ2, whereas the TCR α-chain is uniformly Vα14–Jα281, with a conserved junctional sequence[38,39]. These cells are present throughout the immune system, usually at a low frequency (<5% of all T cells), but they are abundant in the liver. A distinct, CD1d-independent population of NKT cells seems to be abundant at other tissue sites[40]. Conversely, there are CD1d-reactive T cells that do not use the Vα14–Jα281 receptor, but that might be involved in intrahepatic pathology[41]. Whereas the classical NKT cells originate in the thymus[42], an intrahepatic origin has been proposed for other NKT-like cells[43]. The lineage relationships of these other NKT-cell populations are not known, and their identification is complicated by the fact that conventional T cells can express NK-cell markers after activation[44,45].

The function of NKT cells is of great interest. Early on, NKT cells were shown to secrete IL-4, and they might, therefore, be seen as 'anti-inflammatory' lymphocytes[46]. In support of this idea, one well-documented function of these cells is protection from autoimmune disease in non-obese diabetic (NOD) mice, which lack these cells[47,48]. However, *in vivo* analysis indicates that, in the liver, NKT cells have a positive role in host defence. Mice lacking the CD1d molecule have been reported to lack defences against *Mycobacterium tuberculosis* and *Borrelia burgdorferi*[49,50]. The data on anti-protozoan immunity are conflicting, indicating an apparent role

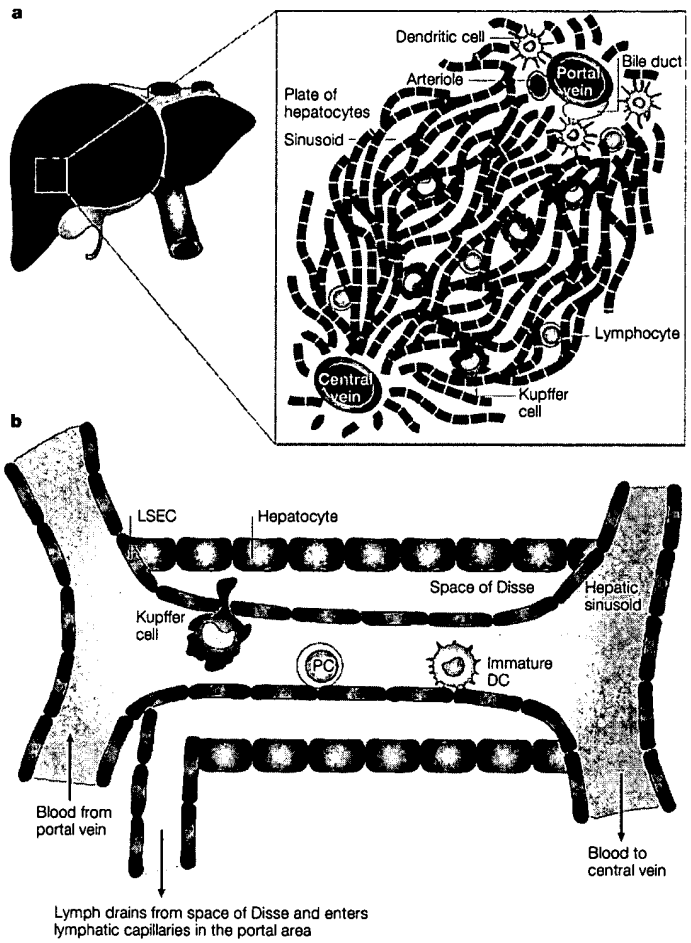

Figure 2 | **The hepatic microenvironment. a** | Diagram showing the structure of a liver lobule. The tissue is organized around vascular bundles, which are known as portal tracts. These contain a branch of the portal vein, an arteriole and a tributary of the bile duct. From the portal tracts, blood flows through a sponge-like anastamosing meshwork of sinusoids that exist between plates of hepatocytes. The sinusoids contain a large population of macrophages, known as Kupffer cells. **b** | Organization of sinusoids. The sinusoid is lined by an endothelium (liver sinusoidal endothelial cells, LSECs) that is fenestrated and lacks a basement membrane. Kupffer cells, lymphocytes (Pit cells, PCs) and immature dendritic cells (DCs) are found in the sinusoids. Kupffer cells exist mainly in the sinusoidal lumen, but they can make direct contact with hepatocytes. The sub-endothelial space, known as the space of Disse, is the region from which hepatic lymph originates.

for CD1d-reactive NKT cells in immunity to trypanosomes[51,52], but no role in immunity to mouse malaria[53]. One complication in the interpretation of these experiments is that the lack of CD1d might cause effects that are not due simply to the lack of NKT cells[54]. Mice that selectively lack the Vα14–Jα281 subset of NKT cells have been created, and these mice are deficient in anti-tumour immunity, which indicates that classical NKT cells have an important role in this process[55,56]. Another tool to study the function of NKT cells is α-galactosyl ceramide (α-GalCer), a molecule derived from marine sponges that causes acute NKT-cell activation, resulting in the synthesis of IFN-γ and IL-4, followed by deletion of the cells[57]. Using α-GalCer, the activation of NKT cells *in vivo* was accompanied by the inhibition of melanoma metastases[58], induction of effective immunity to the liver stages of malaria[59] and suppression of viral-RNA synthesis in HBV-transgenic mice[60]. In summary, there is strong evidence that liver NKT cells promote immunity, but no evidence to implicate these cells in liver tolerance.

The normal resident lymphocytes of the human liver have not been characterized in such detail, but similar to rodent liver lymphocytes, there is an increase in $CD8^+$ T cells relative to $CD4^+$ T cells, and an increase in the number of $CD3^+$ cells that co-express the NK-cell marker CD56 (REF. 22). Some, but not all, of these cells express a TCR in which the Vα24 segment is joined to JαQ, forming a conserved TCR that is analogous to the Vα14–Jα281 receptor on mouse NKT cells[61]. As in the mouse, the caveat must apply that the expression of NK-cell markers by αβ T cells could simply identify activated cells. A subset of normal human liver lymphocytes that express the markers CD2 and CD7 also express messenger RNA encoding recombination-activating gene 1 (RAG1), RAG2 and pre-Tα, which indicates that these cells might be undergoing lymphocyte-receptor gene rearrangement, and which supports the concept that the liver is a site of lymphoid development[62]. However, it is also possible that these mRNAs are expressed aberrantly by haematopoietic stem cells, which are present in human liver[63].

During inflammation, the lymphocyte populations of the liver change. In mice, experimental infection with *Propionibacterium acnes* causes a relative increase in the number of T cells and a corresponding decrease in the number of NKT cells[64], and the same changes are seen in mice with fatty livers[65]. Both of these pathologies sensitize the liver to endotoxin-mediated damage, which indicates that NKT cells might protect against such damage. In line with a tissue-sustaining function of NKT cells, the number of these cells increases during liver regeneration after partial hepatectomy[66]. Infection with lymphocytic choriomeningitis virus results in two phases of T-cell infiltration, involving heavy $CD8^+$ T-cell infiltration of the sinusoids, followed by $CD4^+$ T-cell infiltration of the portal tracts[67]. In humans, liver biopsy material provides a picture of liver lymphocytes during hepatitis. There are increases in the number of activated $CD8^+$ T cells[68] and in the number of $CD4^+$ T cells relative to the number of $CD8^+$ T cells[69]. During infection with HCV, the $CD8^+$ T cells are activated (they express CD45RO) and are located mainly in the sinusoids, whereas the $CD4^+$ T cells are mainly naive (they express CD45RA) and are located in the portal tracts[70]. The number of T cells that express the γδ TCR is also increased during viral hepatitis[71]. Overall, the inflamed liver shows a shift from the resting pattern of abundant NKT cells and an excess of $CD8^+$ T cells over $CD4^+$ T cells towards a more conventional pattern that is characteristic of other inflammatory sites. This is entirely consistent with a role for NK cells in initiating intrahepatic immune responses, but with their numbers then being diluted by the T cells that they recruit.

Interaction of the liver with systemic T cells

In the remainder of this review, I consider the interactions between the liver and conventional CD4+ and CD8+, αβ T cells. Circulating T cells pass through the liver sinusoids and can interact with Kupffer cells and LSECs. Some experiments indicate further that naive T cells might interact with hepatocytes. Antigens that are expressed in the liver might be taken up by immature DCs, and might then be presented to CD4+ and CD8+ T cells, either in lymphoid-tissue aggregates in the portal tracts or in secondary lymphoid tissues. Alternatively, antigens might be recognized *in situ* on LSECs, Kupffer cells and, possibly, hepatocytes. The outcome of antigen recognition in the liver could be full T-cell activation, immune deviation leading to the differentiation of T cells to a suppressive or regulatory phenotype, or abortive activation leading to T-cell apoptosis. In addition, the liver might sequester activated T cells in an antigen-independent manner, and the high apoptotic rate of such cells has given rise to the idea that the liver might be a 'graveyard' for systemic T cells[72]. Inflammation of the liver due to viral hepatitis is accompanied by the upregulation of expression of an extensive panel of T-cell interaction molecules, including intercellular adhesion molecule 1 (ICAM1), MHC class II molecules[73], vascular cell adhesion molecule 1 (VCAM1)[74], co-stimulatory molecules of the B7 family[75] and CD95 (FAS)[76]. These molecules might modify cell trafficking, priming and the induction of tolerance.

Cell trafficking

In most blood vessels, leukocytes flow past the endothelium without forming adhesions. Under inflammatory conditions, selectins expressed by leukocytes interact with addressins expressed by the endothelium to initiate 'rolling', which slows the cells and allows other adhesion molecules to become engaged[77]. *In vivo* microscopy shows that the blood flow in hepatic sinusoids is slow and intermittent, facilitating interactions between blood cells and the endothelium, which might, therefore, be independent of selectin–addressin interactions[78]. LSECs express several adhesion molecules, including a high density of ICAM1 and ICAM2 (REF. 79), and vascular adhesion protein 1 (VAP1)[80]. The reason for this constitutive expression of adhesion molecules is unknown, but one factor might be that LPS from the intestine interacts with Toll-like receptor 4 (TLR4), which is expressed in the liver[81], probably by LSECs[82]. This interaction between LPS and liver cells increases the level of expression of adhesion molecules[83]. The ICAMs engage lymphocyte function-associated antigen 1 (LFA1) on activated T cells, whereas VAP1 binds to unknown ligand(s) on CD8+ T cells and on CD16+ cells, including NK cells. Therefore, the adhesion molecules that are expressed by LSECs give the liver a dual T-cell tropism: for activated T cells in preference to resting T cells, and for CD8+ T cells and NK cells in preference to CD4+ T cells.

In addition to lymphocytes, DCs also traffic through the liver, passing from blood in the sinusoids to the space of Disse, from there through lymphatics in the portal tracts, and ultimately to the hepatic and celiac lymph nodes[84]. The migration of DCs seems to be controlled by chemokines[85,86] (FIG. 4). During experimental infection with *P. acnes*, blood-bourne DC precursors expressing CC-chemokine receptor 1 (CCR1) and CCR5 formed intrahepatic granulomas in response to CCL3. After maturation, the DCs expressed CCR7 and became responsive to CCL21 (secondary lymphoid tissue chemokine, SLC), which promoted their migration to organized lymphoid tissue[87].

Antigen presentation

The liver can present antigen to T cells through several mechanisms. The Kupffer cells form an antigen-trapping system, and the liver is patrolled by 'professional' APCs in the form of immature DCs. In humans, CD45+ cells with high-level expression of MHC class II molecules and a dendritic morphology can be seen in the portal tracts, but these cells are unusual in that they lack expression of CD11C, which is usually found on DCs[88]. In mice, two populations of DC exist, which are distinguished by their expression of CD8α. The DCs that do not express CD8 have been termed 'myeloid' DCs, whereas DCs that express CD8α have been termed 'lymphoid' DCs. However, it is not clear that these cell types originate from distinct lineages[89]. Both types of DC are present in the mouse liver, and both can differentiate into effective APCs *ex vivo*[90]. Trafficking immature mouse DCs pass through the liver, and while they are in the hepatic sinusoids, these DCs are likely to interact with Kupffer cells[91], in part through their expression of lectin-like carbohydrate receptors[92,93]. This interaction might be facilitated by CCL3 synthesized by the Kupffer cells, which communicates with the DCs through their CCR1 chemokine receptors (FIG. 4).

Box 2 | **Experimental models of immune-mediated liver damage**

Injection of the lectin concanavalin A (ConA) into mice causes T-cell-dependent liver damage. This model depends on CD4+ T cells, interferon-γ and natural killer cells. The difficulty in interpreting this model results from the fact that ConA binds many ligands and might have unknown effects in the liver in addition to recruiting and activating T cells[165,166].

The infusion of non-tolerant T cells into a transgenic mouse that expresses an MHC class I molecule on hepatocytes results in transient hepatitis, accompanied by apoptosis of the T cells[103,167]. The difficulty in interpreting this model lies in the difficulty of knowing on which cells the MHC transgene is expressed.

Transgenesis has been used also to express the entire genome of hepatitis B virus (HBV) and of hepatitis C virus in hepatocytes. Such mice develop tolerance to virus-encoded proteins, but the infusion of non-tolerant T cells causes liver inflammation[168,169]. Interpretation of this model is complex, because although the viral antigens are targeted to hepatocytes, cross-presentation might occur on liver sinusoidal endothelial cells, Kupffer cells or dendritic cells. Virus proteins might have evolved to cause immune effects and/or to have direct cytopathic effects, which makes models of this kind both realistic and difficult to unravel. In the HBV-transgenic model, the suppression of virus RNA was due to cytokines, rather than cytotoxic mechanisms.

A simple model of 'pure' T-cell-mediated liver damage has been developed by the injection of specific antigenic peptides into T-cell-receptor-transgenic mice[142,143]. In this model, there are no restrictions on the site of antigen presentation, which makes models that are based on the roles of different antigen-presenting cells difficult to test. Crossing the transgenic mice to a background that lacks death receptor(s) has shown their importance for this kind of liver damage.

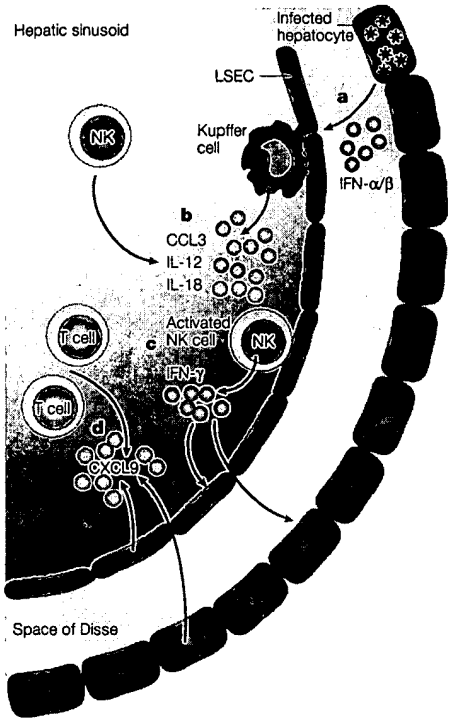

Figure 3 | **The cytokine/chemokine cascade through which NK cells recruit T cells.** The steps are: **a** | many cell types, including hepatocytes, synthesize type 1 interferons (IFN-α/β) in response to virus infection; **b** | Kupffer cells respond to IFN-α and IFN-β by producing CCL3, which recruits natural killer (NK) cells; **c** | NK cells, once activated by the Kupffer-cell products interleukin-12 (IL-12) and, perhaps, IL-18, produce IFN-γ, which causes tissue cells, including hepatocytes and liver sinusoidal endothelial cells (LSECs), to produce CXCL9; **d** | CXCL9 recruits T cells.

An interaction between DCs and macrophages might be important for CROSS-PRESENTATION — for example, of antigen derived from the phagocytosis of apoptotic Kupffer cells[94,95] — and also for priming of the immune response to pathogens.

The *in vivo* function of DCs might lead to effective T-cell priming or to T-cell tolerance. The evidence for tolerance induction by some DCs is clear, and one important distinction between DCs that induce immunity and those that induce tolerance is that the former secrete IL-12, whereas the latter secrete IL-10. As I discuss later, secretion of IL-10 is prevalent in normal liver. Another important issue is whether the DCs that are located in the portal tracts are involved actively in antigen presentation at that site or whether they must traffic to lymph nodes first.

In addition to DCs and Kupffer cells, antigens passing through the hepatic sinusoids encounter LSECs, which form a highly distinctive endothelium. Mouse LSECs express molecules that promote antigen uptake, including the mannose receptor and the scavenger receptor, and molecules that promote antigen presentation, including CD40, CD80 and CD86 (REF. 96). These cells are, therefore, equipped to act as APCs, and they present antigens to both $CD4^+$ and $CD8^+$ T cells (FIG. 5). Despite their expression of co-stimulatory molecules, the most common consequence of T-cell priming by LSECs is tolerance[97,98]. The reason for this paradoxical behaviour is unknown. By contrast, human LSECs express CD40, but they do not seem to express CD80 or CD86 constitutively. These molecules are, however, expressed during inflammation[99]. So, the APC properties of mouse and human LSECs might differ.

The sinusoidal endothelium is fenestrated. Small holes, clustered together to form sieve plates, facilitate the diffusion of metabolites backwards and forwards between the blood and hepatocytes, and scanning electron micrographs have indicated that there are also gaps between the LSECs. This creates the possibility that both naive and previously activated T cells could gain direct access to hepatocytes. This would be a unique situation, because resting naive T cells do not have access to most tissues, which are selectively populated with long-lived memory T cells[100,101]. The extent to which such tissue access occurs in the liver is controversial. The selective accumulation of antigen-specific T cells in the livers of transgenic mice expressing either the HBV genome[102] or a non-self MHC class I molecule[103] is compatible with the idea that such direct access to hepatocytes occurs in the living liver. However, these data are open to alternative interpretations. The HBV antigens could have been subject to cross-presentation, for example by LSECs, whereas the non-self MHC class I molecules could have been ectopically expressed.

T-cell tolerance due to apoptosis

The infusion of non-tolerant $CD8^+$ T cells into transgenic mice that express a non-self MHC class I alloantigen on hepatocytes results in the rapid and selective accumulation of these T cells in the liver. The T cells become activated and might undergo several cell divisions, but ultimately, they die by apoptosis[104,105] (FIG. 5b). In mice that contain antigen-specific TCR-transgenic $CD8^+$ T cells and are injected systemically with soluble antigenic peptide, activation and proliferation of the T cells in lymphoid tissues is followed by the accumulation and apoptosis of these T cells in the liver[25,30]. Finally, transplantation of an allogeneic liver into both humans and mice results in T-cell infiltration, followed by apoptosis of the infiltrating cells[106,107]. To investigate the basis of these observations, naive T cells have been cultured with purified alloantigenic hepatocytes, which results in partial activation of the T cells, followed by their apoptosis. This is believed to be an example of a type of apoptosis known as PASSIVE CELL DEATH (PCD).

In mature T cells, PCD can occur when fully activated T cells are deprived of antigen, or of growth and survival factors such as IL-2. This seems to be a main mechanism for the clearance of expanded clones of T cells after antigen has been cleared during and after an acute infection, whereas FAS-induced apoptosis is less important in this situation[108]. The mechanism of PCD involves loss of mitochondrial membrane integrity,

CROSS-PRESENTATION
The process by which exogenous antigens that are expressed by one cell are processed and presented by MHC class I molecules of another cell. Peptides derived from antigenic proteins are susceptible to this form of presentation, whereas MHC alloantigens are not. Dendritic cells and liver sinusoidal endothelial cells are particularly efficient at cross-presentation.

PASSIVE CELL DEATH
The death of T cells due to activation in the absence of sufficient survival signals, or when antigen is cleared and signals through the T-cell receptor cease.

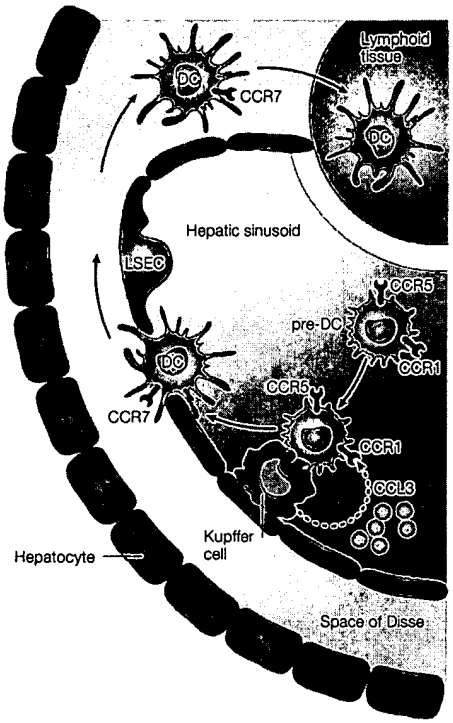

Figure 4 | **Trafficking and interactions of dendritic cells in the liver, controlled by chemokines.** In response to inflammatory signals, Kupffer cells secrete CCL3, which engages CC-chemokine receptor 1 (CCR1) on immature dendritic cells (pre-DCs). Maturation of these cells results in the loss of expression of CCR1 and CCR5, and the expression of CCR7. The DCs become responsive to CCL21, which is produced by lymphatic endothelia and tissue stroma, both in lymph nodes and in the portal tracts. DCs leave the hepatic sinusoids and migrate to lymphoid aggregates in the portal tracts or to lymph nodes. LSEC, liver sinusoidal endothelial cell.

the release of cytochrome *c* and the activation of pro-caspase-9 through apoptotic protease-activating factor 1 (APAF1)[109,110]. Once activated, caspase-9 cleaves and activates pro-caspase-3 and -7, which yields the effector caspases that are responsible for the breakdown of many crucial substrates, including other caspases[111]. In contrast to PCD, ACTIVATION-INDUCED CELL DEATH (AICD) occurs in fully activated T cells and is promoted by IL-2 (REFS 112,113). This process depends on the expression of death receptors, such as FAS and tumour-necrosis factor receptor 1 (TNFR1), and on activation of the death-receptor-initiated caspase cascade, which occurs in part by the downregulation of expression of the inhibitory molecule FLIP (FLICE-like inhibitory protein)[114]. After their ligation, death receptors assemble with adaptor proteins to form a death-inducing signalling complex (DISC), the effect of which is to cleave and activate pro-caspase-8 (REF. 115). Activated caspase-8 cleaves pro-caspase-3, leading to convergence of the PCD and AICD pathways[111].

ACTIVATION-INDUCED CELL DEATH
(AICD). The apoptosis of fully activated T cells, mediated by ligation of death receptors — such as CD95 (FAS), tumour-necrosis factor receptor 1 (TNFR1) and TNF-related apoptosis-inducing ligand receptor (TRAILR) — on their surface.

Experiments in which hepatocytes induce the abortive activation, followed by death, of naive T cells might be examples of PCD. The addition of exogenous IL-2 inhibits the death of T cells that are co-cultured with hepatocytes, which supports the theory that this form of cell death is PCD[105]. Mouse hepatocytes, unlike mouse LSECs, do not express CD80 and CD86 (REF. 116), which indicates that PCD might be induced by the presentation of antigen in the absence of normal co-stimulatory ligands — the same conditions that would cause anergy of a previously activated T-cell clone[117]. A role for PCD due to lack of co-stimulation in liver allograft tolerance *in vivo* was indicated by experiments in which liver-grafted mice were treated with cytotoxic T-lymphocyte antigen 4 (CTLA4)–immunoglobulin, which is an inhibitor of CD80/CD86-mediated co-stimulation. In the CTLA4–immunoglobulin-treated mice, enhanced graft survival was associated with the increased apoptosis of graft-infiltrating $CD4^+$ and $CD8^+$ T cells[118].

If T cells die in the liver by PCD, death receptors such as FAS and TNFR1 would be expected to be irrelevant. One clear example contradicts this and provides evidence in favour of an AICD model. The $CD8^+$ T-cell infiltrate in livers infected with a recombinant adenovirus vector was cleared by a Fas-dependent mechanism[119]. This result argues against a purely passive interpretation of T-cell death in the liver. Such AICD-based models might depend on systemic T-cell activation, because such activation induced by superantigens results in the upregulation of expression of Fas ligand (FasL) in several tissues, including the liver[120].

How can the liver induce both PCD and AICD of $CD8^+$ T cells? The answer might be that AICD depends on full T-cell activation and differentiation. In most of the models that I discuss, T-cell activation is not optimum. Examples include the transgenic expression of non-self MHC molecules on hepatocytes and a liver transplant in the absence of infection. Although the transplant procedure might result in tissue damage and some inflammation, the antigen-presenting system must not be sufficiently perturbed to change to an immunogenic state. Both of these situations, therefore, seem to result in PCD of $CD8^+$ T cells. By contrast, in the adenovirus model, there was evidence for the involvement of Fas and, therefore, of AICD. So, it is probable that the liver kills $CD8^+$ T cells by two mechanisms. Direct recognition of antigen on LSECs, and possibly also on hepatocytes, by naive $CD8^+$ T cells results in partial activation of the T cells, followed by PCD (FIG. 5b). By contrast, recognition of liver antigens by $CD8^+$ T cells in the context of an adenovirus infection results in full T-cell activation, rendering the T cells susceptible to AICD. The role of AICD in the disappearance of $CD8^+$ T-cell infiltrates in other models of viral infection is unexplored, although an unusual variation of AICD might occur in liver $CD8^+$ T cells that are specific for *Listeria monocytogenes*, the clearance of which depends on perforin[121].

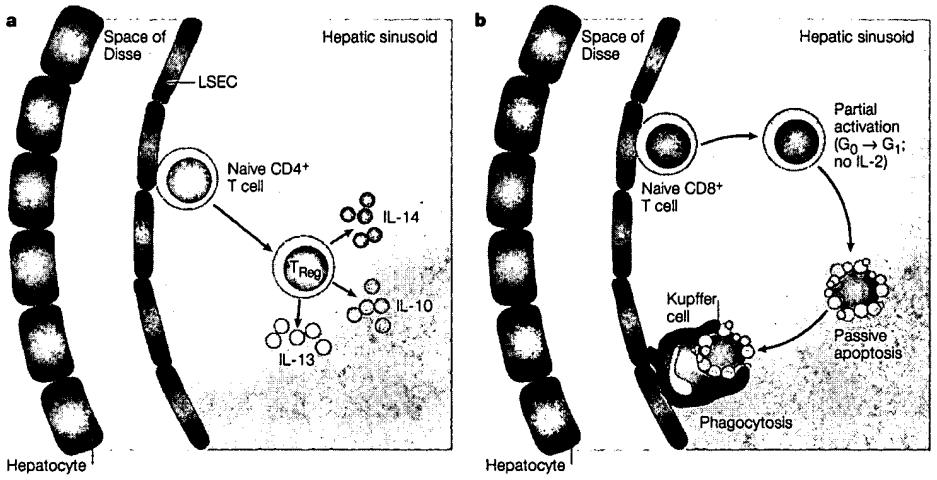

Figure 5 | **Induction of T-cell tolerance by interaction of T cells with liver sinusoidal endothelial cells. a** | Interaction of naive CD4+ T cells with liver sinusoidal endothelial cells (LSECs) results in differentiation of the T cells to a regulatory (T_{Reg}) phenotype. This is probably because LSECs normally produce interleukin-10 (IL-10), which favours the regulatory pathway of CD4+ T-cell differentiation. **b** | Interaction of naive CD8+ T cells with sinusoidal endothelium results in partial activation of the T cells, followed by passive cell death. The reason for this is unknown, but I propose that continuous exposure to trace amounts of intestinal lipopolysaccharide (LPS) results in the differentiation of both LSECs and liver DCs to a state that promotes different forms of T-cell tolerance in CD4+ and CD8+ T cells. The apoptotic CD8+ T cells are then endocytosed by Kupffer cells.

Resting memory T cells seem to be resistant to the pro-apoptotic effects of the liver. So, virus-specific memory CD8+ T cells are found in this site, as are TCR-transgenic memory CD4+ T cells specific for a protein antigen[100,101]. For these cells, the liver might not be a killing field, but rather a hospitable neutral zone.

Immune suppression, regulation and deviation

These two mechanisms to promote CD8+ T-cell apoptosis are not the only tolerogenic processes that are active in the liver. Soluble antigens passing through the sinusoids are taken up by LSECs and presented to both CD8+ T cells and CD4+ T cells. T cells that are primed by LSECs might become activated and undergo proliferation, but they fail to sustain secretion of IL-2 and IFN-γ. CD8+ T cells that are primed by LSECs fail to differentiate into cytotoxic effector cells, whereas CD4+ T cells that are primed by LSECs might differentiate towards an anti-inflammatory (IL-4- and IL-10-secreting) phenotype[97] (FIG. 5a). The local synthesis of IL-10 by T cells primed by LSECs might be important, because IL-10 alters the expression of chemokine receptors by DCs in ways that would be expected to disrupt their homing to lymphoid tissues. Specifically, IL-10 causes the increased expression of CCR5 and the decreased expression of CCR7 by DCs, which renders them less responsive to the lymphoid-tissue chemokine CCL21 (REFS 122,123). The synthesis of IL-10 is prevalent in the liver. In addition to LSECs, mouse liver-derived DCs also preferentially induce the synthesis of IL-10 by CD4+ T cells, in contrast to bone-marrow-derived DCs, which preferentially induce IFN-γ synthesis[90]. In rat liver allografts, an early phase of T-cell apoptosis is followed by the accumulation of CD4+CD45RC^{low} cells that produce IL-13 (REF. 124). So, although liver allografts seem to be protected in the acute phase by apoptosis of host CD8+ T cells, they might survive in the long term due to the presence of host CD4+ regulatory T cells.

What overcomes base-line tolerance?

I have described the liver as an organ in which the antigen-presenting capacity of LSECs and many DCs is biased strongly towards the induction of CD4+ T cells with a regulatory phenotype, whereas both CD8+ T cells that are activated systemically that localize to the liver, and naive CD8+ T cells that first encounter antigen in the liver, are predisposed strongly to undergo apoptosis. The problem is to understand how this default state of tolerance might be reversed to allow priming of T cells to occur and effector responses to be delivered. The solution is not self-evident, but there are some clues.

Type 1 interferons (IFN-α/β) activate a cytokine/chemokine cascade that recruits T cells during virus infections[34,35]. Such IFN synthesis occurs in tissue cells, including hepatocytes, in response to virus infection. In addition, type 1 IFNs induce the synthesis of IL-15, which promotes the survival of CD8+ T cells[125,126]. So, synthesis of type 1 IFNs might be an important event that overcomes liver tolerance and allows an intrahepatic T-cell response to proceed.

A second factor might be the regulation of DC function. The maturation of DC precursors seems to be a two-stage process. Initial maturation results in upregulation of the antigen-processing machinery (including MHC class I and II molecules), expression of co-stimulatory molecules (CD80 and CD86) and the synthesis of IL-10. DCs at this developmental stage prime regulatory T cells preferentially, and their

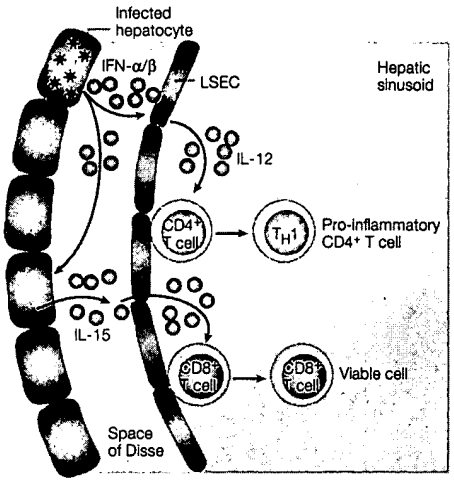

Figure 6 | **An hypothesis to explain how the tolerant state of the liver is reversed, resulting in T-cell priming and immunity.** This hypothesis gives type 1 interferons (IFN-α/β) a central role in the normal liver, and it proposes that chronic exposure to lipopolysaccharide results in the desensitization of sinusoidal endothelial cells to activation signals. These cells present antigen in the context of co-stimulatory molecules, but do not secrete pro-inflammatory cytokines. Therefore, $CD4^+$ T cells become regulatory, whereas $CD8^+$ T cells undergo passive cell death, as shown in FIG. 5. As shown here, under the influence of type 1 IFNs in an infected liver, everything changes. A virus-infected hepatocyte secretes type 1 IFNs, which act on liver sinusoidal endothelial cells (LSECs), causing them to secrete interleukin-12 (IL-12) and promote the differentiation of $CD4^+$ T cells to inflammatory T helper 1 (T_H1) cells. In parallel, type 1 IFNs act on other parenchymal cells, causing them to secrete IL-15, a survival factor for $CD8^+$ T cells. Not shown in this figure is the type 1 IFN-initiated cytokine/chemokine cascade that promotes T-cell recruitment (FIG. 3).

phenotype and functions are markedly similar to those of LSECs. By contrast, the final maturation of DCs results in the synthesis of IL-6, IL-12 and tumour-necrosis factor (TNF), and such DCs prime $CD4^+$ T cells to deliver an immune response[127]. The first maturation step is induced by diverse signals, including exposure to elements of the gut flora[128], whereas the second step is induced by exposure to LPS[129]. I have already considered the effects of intestinal LPS on promoting the expression of adhesion molecules in the liver[83], and this effect indicates that LPS should also promote the final maturation of liver DCs. However, liver DCs prime $CD4^+$ T cells to have a regulatory function[90]. How can this be explained?

I would like to put forward the argument that tolerance-inducing liver DCs are in a state of partial LPS resistance, brought about by continuous exposure to LPS. So, they are refractory to final maturation signals through TLR4 and remain in a state that promotes the differentiation of regulatory T cells. If this is the case, a distinct signal must be required to promote final DC maturation and, thereby, the priming of T cells to deliver effector function. This requirement is met by type 1 IFNs. In addition to its other functions, which have been discussed already, IFN-α promotes DC maturation[130,131], and the type 1 IFNs have been proposed to be a crucial link between innate and adaptive immunity[132].

On the basis of this discussion, I propose the following model. First, the main reason for $CD4^+$ T-cell tolerance induced by antigen presentation in the liver is that both DCs and LSECs respond to the continuous presence of LPS of intestinal origin by becoming unresponsive to TLR4 signals and by assuming a differentiation state that promotes the activation of $CD4^+$ regulatory T cells. This has been shown for LSECs by Knolle *et al.*[133], and I propose that it applies to liver DCs also. Second, the main reason for $CD8^+$ T-cell tolerance in the liver is that liver DCs and LSECs cause partial activation of $CD8^+$ T cells, leading to PCD. Data from my own laboratory show an important role for LSECs in inducing $CD8^+$ T-cell trapping and death[25]. Third, pathogens that elicit a type 1 IFN response promote naive T-cell recruitment, switch the function of liver DCs and LSECs so that they promote the differentiation of $CD4^+$ T cells, and allow full activation and survival of $CD8^+$ T cells (FIG. 6). Thereby, an effective immune response will be delivered. Finally, it follows that pathogens that persist in the liver either suppress type 1 IFN production or evade the effects of type 1 IFNs.

FAS expression and hepatocyte damage

The location of the liver downstream from the huge absorptive surface of the gastrointestinal tract places it in the front line in relation to toxic chemicals, and the liver has evolved the capacity to detoxify potentially dangerous molecules, often through conjugation to glucuronic acid followed by excretion into the bile. However, the uptake of such molecules by hepatocytes poses a constant risk of DNA damage, which could lead to mutation and carcinogenesis. The capacity to induce rapid apoptosis of mutant hepatocytes might explain why hepatocytes express FAS on their membranes. In mice, the Fas signalling pathway is active in these cells, and they are always poised to undergo apoptosis; this can be shown by the *in vivo* injection of Fas-specific antibodies[134] or by the co-culture of hepatocytes with FasL-expressing cells[135]. This capacity to undergo apoptosis is linked to the potential for extensive regeneration, which is manifested as recovery after acute hepatitis, liver regeneration after partial hepatectomy[136] and the restoration of full hepatocyte mass after the transplantation of a 'split liver'[137]. The regeneration of the liver is linked closely to its resident lymphocytes. During the early stages of liver regeneration, there is a rapid, transient increase in the proportion of resident NKT cells[66]. This raises the possibility that, similar to other populations of T cells with a restricted TCR repertoire[138,139], these cells might be involved in the response to tissue damage. However, the role of lymphocytes in liver regeneration is unclear, and NK cells have been proposed to both support[140] and suppress[141] regeneration.

The expression of Fas by mouse hepatocytes places them at risk of acute damage whenever activated lymphocytes accumulate in the liver. In the simplified model of systemic CD8+ T-cell activation induced by the injection of antigenic peptide into TCR-transgenic mice, T-cell accumulation and apoptosis in the liver was accompanied by hepatocyte damage, resulting in histological lesions and elevation of the level of serum aminotransaminases[30,142]. Such damage was reduced in Fas-deficient mice[143], which indicates that FasL — either on the CD8+ T cells themselves or induced by their presence — engages Fas on the hepatocytes. In humans, the situation is more complicated. The basal level of expression of FAS by human hepatocytes is lower than in mice, but it is elevated in inflammatory conditions, including infection with HBV or HCV[144]. This does not seem to be associated with hepatocyte death. In fact, the expression of FAS in the infected liver might be part of antiviral defences during infection with HCV, because it is negatively correlated with liver damage, as assessed by the level of alanine aminotransaminase[145]. FAS expression is also a good predictor of the response to IFN-α therapy[146]. *In vivo*, the limiting factor might be the local expression of FASL. T cells could potentially provide FASL in two ways: by its expression on activated T cells[147,148] or by an immune interaction with hepatocytes in which the ligation of CD40 induces the expression of FASL by the hepatocytes themselves[149]. This might be what is happening when mice are treated with a superantigen, which induces the expression of FasL on many tissues, including the liver[120].

Concluding remarks

The global prevalence of infections of the liver, including malaria, HBV and HCV, makes it important that we understand the distinctive constraints on T-cell immunity in this organ. Vaccines against malaria and HCV are still elusive, and the large number of people who are infected with liver pathogens makes therapy, as well as prevention, an important goal of immunological intervention. Antigen presentation by LSECs, and possibly by hepatocytes, might frustrate efforts to prime an effective immune response, and pro-apoptotic mechanisms might eliminate activated liver-specific CD8+ T cells. Highly activated T cells are dangerous in the liver, because hepatocytes express the FAS death receptor and are susceptible to immunopathology. In the face of these obstacles, it is highly desirable that we devise strategies to modify the natural history of such chronic infections by manipulating the immune system to deliver protective responses without unacceptable immunopathology.

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Online links

DATABASES

The following terms in this article are linked online to:

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APAF1 | B220 | caspase-8 | caspase-9 | CCL3 | CCL21 | CCR1 | CCR5 | CCR7 | CD1d | CD2 | CD7 | CD8α | CD11C | CD25 | CD40 | CD56 | CD69 | CD80 | CD86 | CD95 | CTLA4 | CXCL9 | FLIP | ICAM1 | ICAM2 | IFN-α/β | IFN-γ | IL-2 | IL-2Rβ | IL-4 | IL-6 | IL-10 | IL-12 | IL-15 | LFA1 | NK1.1 | RAG1 | RAG2 | TLR4 | TNF | TNFR1 | VAP1 | VCAM1

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REVIEW

Dendritic cells and immune regulation in the liver

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Hepatic dendritic cells (DC) unquestionably play important roles in the induction and regulation of immune responses. Due to their paucity, functional characterisation of these important antigen presenting cells has been slow but use of DC growth factors (in particular GM-CSF and Flt3L) that markedly enhance their numbers has proved helpful in furnishing adequate study material. While there is growing evidence that DC function is affected in the pathogenesis of liver disease, most work to date has been performed on non-hepatic DC. Increasing knowledge of hepatic DC biology is likely to improve our understanding of disease pathogenesis and resistance to and therapy of liver disease.

INTRODUCTION

The liver is an important site of infectious, parasitic, autoimmune, and malignant diseases. Immune responses and their modulation within the liver are critical to the outcome of these conditions and also in liver transplantation. The inherent tolerogenicity of the liver, including its possible role in oral tolerance, poses important questions about how immune reactivity in the liver is regulated.[1] Increasing attention has focused on antigen presenting cells (APC) and the critical roles that they play in both innate and adaptive immunity. APC exist in several forms within the liver and exhibit a spectrum of abilities to capture, process, and present antigen (Ag) to immune effector cells. Although rare, dendritic cells (DC) are the most highly specialised APC, with ability both to instigate and regulate immune reactivity. In addition, DC are well equipped to migrate from peripheral tissue sites such as the liver to regional lymphoid organs, where they present Ag to T cells. In the normal steady state, these events may be important in the maintenance of self tolerance. It is now recognised that the microenvironment in which APC develop or are activated influences their function and their effects on T cell populations. Furthermore, different DC subsets have been identified that exhibit distinct functional capabilities. Progress in uncovering the properties of liver DC has been slow but the recent surge of interest in DC biology and technological advances in their isolation and characterisation have brought these cells to centre stage in the quest for a fuller understanding of immune regulation within and beyond the liver.

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LIVER APC POPULATIONS COMPARED

The liver contains several types of APC (fig 1). Liver sinusoidal endothelial cells (LSEC) line the sinusoids and have a distinct morphology in comparison with vascular endothelial cells that line arterial branches, and central and portal veins.[1 2] In contrast with vascular endothelia, LSEC do not express CD31 (PECAM-1, pgIIa endothelial cell adhesion molecule), which is expressed at tight junctions of vascular endothelia, but exhibit higher constitutive levels of CD54 (ICAM-1, intercellular adhesion molecule 1) and CD106 (VCAM-1, vascular cell adhesion molecule).[1 2] Also, LSEC have fenestrations up to 100 nm in diameter although passage of particles through these openings is selective. Thus although particles as small as 15 nm fail to enter, lymphocytes can access the space of Disse between the lumen of the sinusoids and hepatocytes. Extracellular matrix and hepatic stellate cells are located in this area. Hepatocytes have been reported to act as APC in certain situations although they are not considered to be primary mediators in immune regulation within the liver.[1 3] Kupffer cells (KC), the resident macrophages of the liver, patrol the portal venous system via the sinusoidal lumen and can adhere to LSEC, occasionally causing temporary obstruction of blood flow through the sinusoid (fig 1).[4 5] In normal liver, hepatic DC typically reside only around portal triads[6–8] and, like DC in other peripheral sites, are able to efficiently capture, process, and transport Ag to regional lymphoid tissues. All three APC (LSEC, KC, DC) internalise Ag by phagocytosis, receptor mediated endocytosis, or pinocytosis but their phenotypes differ considerably.[1 2 9] LSEC and KC express major histocompatibility complex (MHC) Ags, costimulatory and adhesion molecules, and make interleukin (IL)-1 and interferon γ (IFN-γ), suggesting that these cells are at a relatively mature stage.[1 2 9 10] Freshly isolated hepatic DC on the other hand are predominantly immature cells, expressing surface MHC but few costimulatory

Abbreviations: Ag, antigen; APC, antigen presenting cells; BM, bone marrow; CC and CXC, chemokines; CCR and CXCR, chemokine receptors; DC, dendritic cell; ECM, extracellular matrix; Flt3L, fms-like tyrosine kinase 3 ligand; GM-CSF, granulocyte macrophage-colony stimulating factor; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; KC, Kupffer cell; IL, interleukin; IFN-γ, interferon γ; LSEC, liver sinusoidal endothelial cells; MHC, major histocompatibility complex; NPC, non-parenchymal cells; PALT, portal tract associated lymphoid tissue; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; TGF-β, transforming growth factor β; TNF-α, tumour necrosis factor α.

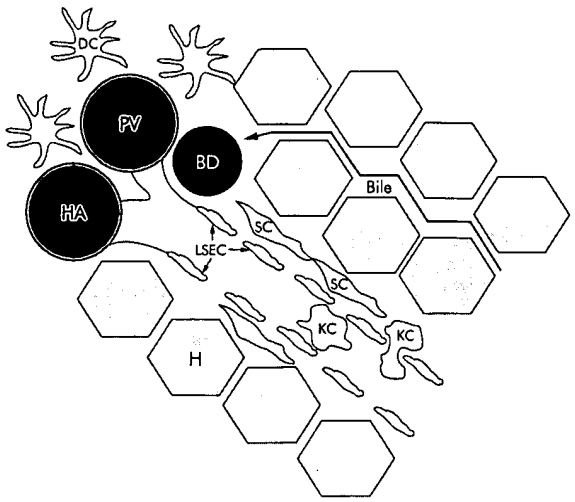

Figure 1 Anatomy of sinusoids. The area between the LSEC and hepatocytes, where extracellular matrix and stellate cells reside, is called the space of Disse. Kupffer cells and other immune cells are believed to extravasate through the LSEC fenestrations into the parenchyma. DC normally reside only in the portal areas. BD, bile duct; DC, dendritic cell; H, hepatocyte; HA, hepatic artery; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cell; PV, portal vein; SC, stellate cell.

molecules necessary for T cell activation.[11–13] Compared with more mature bone marrow (BM) derived or spleen DC, they stimulate naïve allogeneic T cells only poorly.[13–15]

ROLE OF THE LIVER MICROENVIRONMENT AND HEPATIC DC IN TOLEROGENICITY

The immature phenotype of resident hepatic DC coupled with the inherently unique liver microenvironment potentially makes these APC different from DC in other tissue sites (that is, BM, spleen). Although not considered to be an immune privileged site, such as the anterior chamber of the eye or the testis, there are marked similarities between the cytokine milieu of the liver and these other sites. KC and LSEC constitutively express the anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF-β) that are upregulated on stress, while hepatocytes secrete IL-10 in response to autocrine and paracrine TGF-β.[1 2 16 17] Lipocytes, another liver specific cell population that includes Ito and stellate cells, also express increased TGF-β on activation or stress.[16] These cytokines not only affect T helper (Th) T cell differentiation directly (skew to Th2) but also can confer tolerogenicity on DC and other APC by inhibiting their maturation and T cell stimulatory function.

> "There is now much evidence that DC can be rendered tolerogenic"

Although mature DC, rich in surface MHC and costimulatory molecules, are potent stimulators of immune (T cell) function, there is now much evidence that DC can be rendered tolerogenic. Thus exposure of replicating DC progenitors to IL-10 or TGF-β[18] generates DC that are suppressive or tolerogenic. Steinbrink and colleagues[19] showed that culture of immature blood derived human DC with IL-10 inhibited their maturation. Similar results have been obtained with DC transduced with either IL-10 or TGF-β.[20 21] Lack of adequate costimulatory molecule expression, either due to immaturity or exposure to costimulatory pathway blocking agents, can also result in tolerogenic DC, as shown in both allograft[22] and autoimmune disease[23] models.

PHENOTYPE OF HEPATIC DC

Many different markers have been used to identify rodent and human DC, including those that are species specific (table 1). While none are specific to hepatic DC, variations occur in the level of expression of certain markers compared with others. CD11c is a common but not universal marker for DC detection in the murine system. In addition, other markers, such as CD205, have been used by different groups to identify specific murine DC subsets. The two principal subsets identified in mouse liver as well as in lymphoid tissue are the "so-called" myeloid ($CD8\alpha^-CD11b^+$) and lymphoid related ($CD8\alpha^+CD11b^-$) subsets of DC. These DC are distinguished by their reciprocal expression of CD8α and CD11b and were thought initially to have distinct lineage and functions.[24 25] Recent evidence has shown that these subsets derive from a common precursor and that rigid lineage affiliations between subsets may not exist.[26–28] Plasmacytoid DC or type 1 IFN producing cells (a unique cell type of the haematopoietic system) have recently been identified in mouse lymphoid tissues.[29–31] These DC are $CD11c^+CD11b^-CD19^-B220^+$ and $Gr1^+$ and may play crucial roles in antiviral immunity. Whether they are present in normal liver has yet to be determined.

DC have been generated in vitro from mouse liver stem/progenitor cells in response to granulocyte macrophage-colony stimulating factor (GM-CSF). These liver derived DC progenitors[32 33] are distinct in phenotype from DC freshly isolated from normal liver and are $CD11c^{lo}CD24^+CD44^+$. Maturation of DC is associated with upregulation of MHC II, CD80, and CD86, with CD205 being an additional marker used by some groups. Lu and colleagues[34] have also shown that culture of normal murine hepatic non-parenchymal cells (NPC) with IL-3 and CD40L yields a unique population of DC-like cells that are $CD205^{hi}CD11c^-B220^+CD19^-$.

Less diversity has been reported to date for DC markers in the rat and human. OX62, an integrin molecule, is commonly used to detect rat DC.[35–37] As in mice, maturity is monitored by surface expression of the CD28/CTLA4 ligands CD80 and CD86. Two distinct populations of mature rat hepatic DC have been identified: (1) $ED1^+ED2^-OX6^-$ and (2) $ED1^-ED2^-OX6^+$. In humans, DC are commonly MHC II^+ and deficient in CD28/CTLA4 ligands while in an immature state. Prickett and colleagues[7] found that human liver DC were also $CD45^+CD11a^+CD18^+$.

Thus it can be seen that there are similarities and disparities among DC populations. Common features to all three species include the lack of or low expression of MHC II and CD28/CTLA4 ligands on immature DC that are increased on maturation. CD11c and OX62 are generally considered the definitive markers for mouse and rat, respectively.

ENUMERATION OF HEPATIC DC

The normal murine liver, one of the larger visceral organs, has a relatively high total interstitial DC content, about 2–5-fold greater than that of other parenchymal organs, such as the kidney or heart.[38] However, when the density of MHC II^+ DC between these organs is compared, the liver ranks as the lowest.[38]

Specific DC populations, such as myeloid and lymphoid related subsets, studied in other tissues[24 39 40] (table 1), can be found in normal mouse livers. Previous studies have shown that these subpopulations constitute a low percentage of the total tissue specific DC population. The relative proportions of these two subsets in the liver are similar to those seen in other tissues.[12 24 39 40] Each population constitutes 1% of the total normal liver NPC population.[12]

Liver DC can be isolated from NPC by collagenase digestion followed by metrizamide density centrifugation.[12 15 41] Although the total number of DC in the liver is greater than that of other parenchymal organs, there are still few cells to work with in comparison with lymphoid tissue. This paucity of cells

Table 1 Phenotype of liver dendritic cells

Species	Maturation status	Markers	Comments (ref)
Mouse	Immature	$CD11c^+CD40^{lo}CD80^{lo}CD86^{lo}MHC\ II^{lo}$	2 subsets:[1 14 41 101]
		(1) $CD8\alpha^-CD11b^+$	(1) Myeloid related[12 102]
		(2) $CD8\alpha^+CD11b^-$	(2) Lymphoid related
		$B220^-CD11c^+CD205^-F4/80^-$	Yoneyama[65]
		$CD205^+OX2^+$	Gorczynski,[37] Drakes,[43] Gorczynski [103]
		$CD11b^+CD24^+CD44^+CD45^+CD11c^{lo}CD16/3\ 2^{lo}\ CD40^{lo}\ CD80^{lo}$ $CD86^{lo}CD205^{lo}F4/80^{lo}$	Generated from liver progenitor cells with GM-CSF; called liver derived DC progenitors.[32 33]
	Mature	$CD11c^+MHC\ II^{hi}CD86^{hi}$	
		$CD11c^+CD54^+CD205^+MHC\ II^+CD11b^{mod}\ CD86^{mod}CD11a/CD18^{mod}$ $B220^-CD3\varepsilon^-Gr1^-$	Yoneyama[65]
	Other	$CD205^{hi}\ B220^+CD11c^-\ CD19^-$	Generated from liver progenitor cells with IL-3 and CD40L. Ig gene rearrangement occurs but no surface expression. Activate then subsequently induce apoptosis of T cells.[34]
Rat	Immature	$MHC\ II^+ANAE^-FcR^-$	ANAE=α-naphtylacetate esterase=a non-specific esterase; FcR=Fc receptor[53]
	Mature	$MHC\ I^{hi}MHC\ II^{hi}CD54^+OX62^+$	Brenan,[36] Matsuno,[56] Saiki[92]
		(1) $ED1^+ED2^-OX6^+$, (2) $ED1^-ED2^-OX6^+$	2 subsets of $OX62^+$ cells[104]
		$MHC\ II^+CD54^+OX62^+CD90^+CTLA\text{-}4\ Counter\text{-}receptor^+$	Chen-Woan[35]
		$CD4^+$	Variable expression is common in $MHC\ II^+$ DC and peripheral tissues of rat[51 105]
Human	Immature	$CD11a^+\ CD45^+MHC\ II^+$	Prickett[7]
		$CD83^{lo}CD86^{lo}MHC\ II^{lo}$	Ninomiya[84]
	Mature	$CD200^-$	Goddard[106]
		$CD83^+CD86^+$	Goddard[107]

DC, dendritic cell; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; MHC, major histocompatibility complex.

is especially evident if a specific DC subset is sought. Administration of recombinant human fms-like tyrosine kinase 3 ligand (Flt3-ligand, Flt3L), an endogenous haematopoietic growth factor, markedly increases the total number of hepatic DC.[12] Furthermore, the yield can be further increased by overnight culture of the isolated DC progenitors with GM-CSF. Under such culture conditions, the percentage of both $CD8\alpha^-$ and $CD8\alpha^+$ DC can be increased to 10–15% of the total NPC population.[12]

The phenotype of the DC obtained from Flt3L mobilised mice resembles that of DC isolated from normal liver and in situ.[12 15 33 41–43] Drakes and colleagues[43] showed that administration of Flt3L did not change the phenotype of freshly isolated hepatic DC, as defined earlier. These Flt3L treated DC, on culture with GM-CSF and IL-4 or exposure to a maturation inducing stimulus, such as extracellular matrix (ECM) protein, increased their surface costimulatory molecule expression and T cell allostimulatory activity.[33 43–45]

> "The leucocyte content of the liver and its DC constituency in particular, appear to play an important role in transplant outcome"

The leucocyte content of the liver and its DC constituency in particular, appear to play an important role in transplant outcome. Thus when donor hepatic leucocytes are either drastically reduced[46–48] or greatly augmented,[49 50] a switch from tolerance to rejection occurs in murine liver transplantation. In the case of donor leucocyte depletion, transplant tolerance can be restored by replacement of donor leucocytes.[47] Thus a balance appears to exist between the number of donor hepatic DC and liver tolerogenicity.

APC FUNCTIONS OF HEPATIC DC

Phagocytosis

Early studies showed that intravenous administration of colloidal carbon[8 51 52] or antibody coated human red blood cells[53] did not result in phagocytosis by DC. It was speculated that liver DC, unlike KC and LSEC,[2 54 55] did not phagocytose these particles in vivo. However, more recently, elegant studies in the rat by Matsuno and colleagues[56 57] have shown that carbon laden DC localise in the coeliac nodes within two hours of intravenous administration of carbon particles. Furthermore, it was determined that immature DC were the major population of particle laden cells that entered the hepatic lymph. It was suggested that these phagocytic DC were recruited from the systemic circulation and were not part of the resident DC population. Interestingly, Iyoda and colleagues[58] have reported that in mice, only the liver resident $CD8\alpha^+$ DC subset exhibits phagocytic properties in situ.

T cell stimulation

Murine liver DC progenitors cultured overnight with or without GM-CSF stimulate naïve allogeneic T cells.[14 15 49] Abe and colleagues[13] observed that the allostimulatory activity of immature liver derived DC for memory T cells was not affected by administration of proinflammatory cytokines such as tumour necrosis factor α (TNF-α) or IFN-γ. However, addition of Ag (that is, viral antigen; keyhole limpet haemocyanin) to immature hepatic DC induced upregulation of MHC II, costimulatory molecules, and T cell allostimulatory activity. Khanna and colleagues[14] found that although cultured immature mouse liver derived DC were weak stimulators of allogeneic naïve T cells in vitro, their in vivo administration to allogeneic recipients resulted in selectively increased IL-10 production within secondary lymphoid tissue. By contrast, mature BM derived DC elicited increased IFN-γ but not IL-10 production. Immature hepatic DC therefore resemble freshly isolated immature respiratory tract DC that poorly stimulate allogeneic T cells and selectively induce Th2 responses.[59] These features of liver derived DC are consistent with hepatic "tolerogenicity" and may play a role in immune response deviation following liver transplantation.

There is as yet little documented information on the T cell stimulatory ability of purified freshly isolated human liver DC. Based on their immature phenotype in situ[60] (including lack of CD86) and the known properties of circulating peripheral blood DC with an immature phenotype,[61] it is likely however that these cells are weak allostimulators.

DC isolated from lymph

Matsuno and colleagues[56] have surveyed and analysed rat hepatic DC after they have exited the liver and entered the lymphatic circulation. By selective lymphadenectomy, it is possible to directly anastomose peripheral lymphatics to the thoracic duct, allowing draining cells to circumvent lymphoid tissues.[62] Thus non-lymphoid cells in peripheral lymph can be collected from the thoracic duct. Removal of coeliac nodes allowed enrichment of the lymphatics with hepatic DC, leading Matsuno and colleagues[62] to speculate that the liver is perhaps the greatest source of lymph from the gastrointestinal tract. The particle laden DC that entered the lymph were found to be non-phagocytic, even though they appeared immature cytologically. Furthermore, they were found to be strong T cell allostimulators. It has been suggested that these DC are in the early stages of maturation. Little is known of the activation, maturation, and migration of hepatic DC subsequent to Ag uptake.

Portal tract associated lymphoid tissue (PALT)

Portal lymphoid follicles were described in chronic active hepatitis C as early as 1992.[63 64] These areas of B and T cell interactions exhibit many histological features classic to lymphoid follicles. More recently, Yoneyama and colleagues[65] have identified DC-T cell interactions within these specialised areas of the liver. On infection with *Propionibacterium acnes*, granulomas form within the liver. DC are mobilised to these sites and can be found to (1) traffic to the hepatic LN; (2) remain in the developing sinusoidal granuloma; or (3) associate with immunoresponsive cells (B and T cells, DC) in a distinct area near the portal triad, termed the PALT by Yoneyama *et al*.

> "Portal inflammation and PALT development have been identified in primary sclerosing cholangitis"

This lymphoid tissue-like area comprises B cell follicles with follicular DC (not BM derived DC but DC specialised for the presentation of Ag captured in immune complexes) interspersed throughout the follicles. $CD4^+$ T cells were found to localise between B cell follicles, but not within these structures, unlike the broad distribution seen within sinusoidal granulomas. Surrounding the B and T cell areas were macrophages. In patients with hepatitis C virus infection, plasma cells and B cells are also found in association with DC within hepatic portal areas, as in lymphoid tissue.[66] Similarly, portal inflammation and PALT development have been identified in primary sclerosing cholangitis (PSC).[67 68] CCL21 (secondary lymphoid chemokine), a lymph node associated chemokine, is upregulated on $CD34^+$ vascular endothelium of PALT. Expression of CCL21 recruits $CCR7^+$ cells that commonly include DC and naïve T cells.[69 70] These findings suggest that there may be important immune cell interactions occurring within PALT, perhaps circumventing the need for DC migration to lymphoid tissue.

Liver derived DC progenitors

In order to generate DC from normal liver, Lu and colleagues[33] applied a procedure introduced for the propagation of DC from murine blood or BM. Inaba and colleagues[71 72] first showed that culture of normal mouse BM cells with GM-CSF resulted in the propagation of DC. Similarly, culture of liver NPC yielded a population of replicating DC progenitors.[33] These immature DC exhibited classic veiled morphology, high surface expression of CD45, CD11b, CD24, and CD44, moderate to low expression of CD11c, CD16/32, CD54, CD205, and F4/80, and low expression of the costimulatory molecules CD40, CD80, and CD86. Furthermore, these cells were resistant to typical DC maturation inducing stimuli, such as the proinflammatory cytokines IFN-γ and TNF-α. Extended culture failed to upregulate MHC II or costimulatory molecules. Instead, these cells matured in response to ECM proteins, such as collagen type I[13 33 44 45] (with which DC are associated spatially in normal liver), losing their phagocytic ability and gaining the ability to stimulate naïve allogeneic T cells.

A novel population of mouse liver derived DC-like cells has been propagated in response to IL-3 and CD40L.[34] These cells have a phenotype and function distinct from typical immature or mature myeloid or lymphoid related mouse DC. Ig rearrangement occurs within these cells without surface expression of Ig molecules. Furthermore, they have a distinct pattern of surface markers and maintain a DC-like morphology. These $CD205^{bright}CD11c^-B220^+CD19^-$ cells activate T cells and promote their apoptosis. Lu and colleagues[34] also showed that a T regulatory type 1 cytokine expression pattern was induced by these DC.

Chemotaxis

Migration of DC to and from peripheral tissue depends on the production of chemokines (CC and CXC) and expression of specific chemokine receptors (CCR and CXCR). Because leucocyte migration is a key event in infection and inflammation, chemokine biology is rapidly becoming an important area of study in relation to elucidation of DC function. Most chemokine receptors are promiscuous and can ligate a variety of different chemokines.[73–75]

> "Because leucocyte migration is a key event in infection and inflammation, chemokine biology is rapidly becoming an important area of study in relation to elucidation of DC function"

In the case of hepatic DC, few studies have been conducted regarding specific chemokine and receptor expression. Drakes and colleagues[76] showed that immature and mature liver derived DC exhibited similar chemokines and receptors, although with differing levels of expression. Expression was similar to that detected on BM derived DC. As determined by the RNase protection assay, the chemokine most strongly expressed by both immature and mature liver derived DC was CCL5 (RANTES, regulated upon activation, normal T cell expressed and secreted). However, CCL3 (MIP-1α, macrophage inflammatory protein 1α), CXCL1 (MIP-2), and CCL2 (MCP-1, monocyte chemoattractant protein 1) were also expressed by these liver derived DC. Receptors CCR1 and CCR2 were expressed at comparable levels on these liver DC. CCL5 and CCL3 are among the various chemokines that bind CCR1 while CCL2 binds CCR2. CCL3 expression was greatly enhanced on liver DC maturation and stimulation by bacterial lipopolysaccharide or naïve allogeneic T cells also induced chemotaxis of mature liver derived DC.

Shields *et al* found that CCR5, for which CCL3 is a ligand, is important in T cell recruitment in both hepatitis C virus (HCV) infected and normal livers.[77] Goddard *et al* similarly observed the importance of CCR5 in T lymphocyte recruitment during the inflammatory response in human liver transplantation.[78] The presence of this receptor on T cells coupled with the production of CCL3 by resident liver cells implies the existence of DC-T cell interactions within the liver under normal and inflammatory conditions. Further studies are needed to assess the role of chemokines and their receptors in the regulation of hepatic DC migration and function.

HEPATIC DC AND LIVER DISEASE

Viral hepatitis

Both hepatitis B and C viruses (HBV and HCV, respectively) are major health concerns as these are not only infectious diseases with distinct pathogeneses but are also major prognostic factors for hepatocellular carcinoma (HCC). Although some studies

have investigated the role of resident liver DC in defence against these viruses, there is still much to understand.

"A general agreement in the literature is the existence of dysfunctional DC in both HBV and HCV infection"

A general agreement in the literature is the existence of dysfunctional DC in both HBV and HCV infection. HBV transgenic mice that express HBV Ag are used as a model for chronic HBV carriers. These mice show low immune efficiency, as defined by decreased overall specific antibody responses and lowered DC allostimulatory capabilities.[79 80] In one study, it was found that defective splenic DC had low costimulatory molecule expression and low IL-12 production.[60]

Peripheral blood derived DC from chronic HCV patients show impaired maturation.[81 82] They fail to respond to TNF-α (that typically induces DC maturation) and are poor T cell allostimulators. These cells also show decreased production of bioactive IL-12 p70.[81] Less aggressive HCV infection is associated with inflammation, confined mainly to portal areas where hepatic DC generally reside. In contrast with reports of increased immature blood DC in chronic HCV patients, electron microscopy and surface marker expression have identified the portal infiltrate DC as phenotypically mature.[66] These DC are also associated with the formation of new lymphatic capillaries within chronic hepatitis C livers.[66] Thus Galle *et al* speculate a critical role for DC in mediating the HCV disease state based on increased lymphatic drainage and the association of DC with these sites.

One of the chemokines present in portal areas during HCV infection is CCL3.[77] This chemokine is produced by T cells, macrophages (KC), and fibroblasts, and attracts DC as well as T cells. Other chemokines present in the portal area at sites of piecemeal necrosis in patients with chronic hepatitis C include CCL5 and DC-CK1, which have been correlated with an active immune response against HCV.[83] In fact, DC-CK1 is found in the PALT. It is possible that the production of these cytokines aides DC-T cell interactions.

Hepatocellular carcinoma (HCC)

A prerequisite for effective immune responses against tumours is the need for cells that recognise, process, and present tumour Ag. DC are considered promising biological therapy agents for cancer treatment. In patients with HCC, there is evidence that immature DC with maturation defects are the predominant type of peripheral blood DC.[84] Circulating DC show reduced expression of HLA-DR and IL-12 and reduced endocytotic and allostimulatory capacity.[85] Additionally, these DC remain immature in the presence of high levels of inflammatory cytokines that normally induce DC maturation.[84]

"DC are considered promising biological therapy agents for cancer treatment"

By contrast, it has been reported that activated CD83$^+$ DC are increased in the peripheral blood of HCC patients compared with normal patients and patients with liver cirrhosis.[85] However, total DC are reduced in the livers of HCC subjects and not localised to cancer nodules.[85] Importantly, it had been shown that administration of Flt3L can drastically reduce the number of hepatic metastases in experimental animals.[86] Tumour borders exhibited increased infiltration with both DC and T cells as well as increased apoptotic bodies. Thus DC may have an important role in surveillance and clearance of tumour cells in liver cancer.

Granulomatous liver disease

Recent studies have revealed DC recruitment to hepatic sites of experimental granulomas. Yoneyama and colleagues[65] observed CD11c$^+$F4/80$^-$B220$^-$ DC in *P acnes* induced granulomas in the perisinusoidal space. These DC later interacted with T cells in the PALT.

Autoimmune diseases

Patients with primary biliary cirrhosis (PBC) have dysfunctional DC with increased production of nitric oxide and lowered allostimulatory capability.[61] The number of DC present in portal tracts is greater in PBC patients compared with HCV patients.[60] Kaji *et al* also found that these CD86 positive DC appeared to be more relevant in the earlier stages of PBC as they disappeared from the liver at later stages.

TRANSPLANTATION

The immunobiology of liver transplantation has long been a field of intense study as it may provide valuable insight into the mechanisms underlying transplant tolerance. Liver transplant patients are known to achieve graft acceptance without continued immunosuppressive drug therapy. Moreover, graft failure due to chronic rejection is rare compared with other types of organ transplantation. Furthermore, liver transplantation can protect other organ grafts from the same donor transplanted in conjunction with the liver. Pigs, mice, and some rat strain combinations will accept liver allografts across MHC barriers without immunosuppressive therapy. This acceptance may be lost by removal of donor leucocytes prior to liver transplant[46 47] while replacement of donor leucocytes abrogates rejection.[47] These findings suggest that donor leucocytes, that include DC, have the capacity to modulate host anti-donor immune reactivity.

"Donor leucocytes, that include DC, have the capacity to modulate host anti-donor immune reactivity"

There are several concepts that attempt to explain the comparative privilege of liver allografts. Starzl *et al* have proposed the theory of microchimerism (two way silencing of immune reactivity, linked to deletion of alloreactive T cells) to explain promotion of tolerance induction.[22 87 88] Microchimerism is the persistence of donor haematopoietic cells within both lymphoid and non-lymphoid tissues of the host. Significantly, donor derived DC can be propagated from the blood or BM of liver transplant recipients.[89 90] In mice, this is achieved in liver recipients that accept these grafts without immunosuppression and that develop donor specific tolerance, but not in mice that acutely reject heart grafts from the same donor strain.[89]

The liver is a haematopoietic organ and thus compared with other transplanted organs may have an advantage in being a continuous source of donor haematopoietic stem/progenitor cells. Many circulating haematopoietic cells also take up residence in the liver. In addition, three hepatic stem cell candidates have been described to date: fetal progenitor bipotential hepatic stem cells, adult hepatocytes, and oval cells—a type of non-parenchymal pluripotent hepatic stem cell.[91] The existence of these liver stem/progenitor cells suggests that a hepatic progenitor cell exists for the production of liver specific DC in situ. Importantly, donor interstitial DC appear to self replicate in rat liver graft recipients.[92] These donor derived immature DC may promote donor specific tolerance induction.

It has been argued on the other hand that comparatively large numbers of donor leucocytes present in liver allografts cause overstimulation or "abnormal" early activation of recipient T cells that leads to their exhaustive proliferation and deletional tolerance.[48]

"Conceivably, donor DC may play a role in inducing apoptosis in host T cells via death ligand receptor pathways"

There are many potentially important mechanistic roles for the hepatic DC in determining the outcome of transplantation. Alloreactive host T cell apoptosis in experimental liver transplantation is associated with tolerance whereas less apoptosis is seen with rejection.[93-95] Conceivably, donor DC may play a role in inducing apoptosis in host T cells via death ligand receptor pathways.[96] It has been shown that there may be such a role for tissue resident migratory DC in immune privileged sites, such as the anterior chamber of the eye[97] or testis.[98] Neutralisation of IL-12 produced by liver resident DC and other APC in murine livers transplanted from Flt3L treated donors (that are rejected acutely) restores long term allograft survival and enhances alloreactive T cell apoptosis.[99] This suggests that suppression/inhibition of donor DC function promotes tolerance induction. The immature state of normal liver derived DC, associated with failure to provide adequate costimulation, may be important in inherent liver tolerogenicity. The immature state/absence of costimulation can also be achieved using immunomodulatory agents, such as IL-10[19] or CTLA4-Ig.[23] Administration of liver DC progenitors prior to transplantation has been shown to increase allograft survival, although not to induce tolerance.[34 100] It remains to be determined whether in a clinically relevant large animal (primate) model, coadministration of immature donor DC with appropriate pharmacological agents or biological immunosuppressants that inhibit their maturation and those of recipient DC would promote the induction of organ transplant tolerance.

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Local control of the immune response in the liver.

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The physiological function of the liver--such as removal of pathogens and antigens from the blood, protein synthesis and metabolism--requires an immune response that is adapted to these tasks and is locally regulated. Pathogenic microorganisms must be efficiently eliminated while the large number of antigens derived from the gastrointestinal tract must be tolerized. From experimental observations it is evident that the liver favours the induction of tolerance rather than the induction of immunity. The liver probably not only is involved in transplantation tolerance but contributes as well to tolerance to orally ingested antigens (entering the liver with portal-venous blood) and to containment of systemic immune responses (antigen from the systemic circulation entering the liver with arterial blood). This review summarizes the experimental data that shed light on the molecular mechanisms and the cell populations of the liver involved in local immune regulation in the liver. Although hepatocytes constitute the major cell population of the liver, direct interaction of hepatocytes with leukocytes in the blood is unlikely. Sinusoidal endothelial cells, which line the hepatic sinusoids and separate hepatocytes from leukocytes in the sinusoidal lumen, and Kupffer cells, the resident macrophage population of the liver, can directly interact with passenger leukocytes. In the liver, clearance of antigen from the blood occurs mainly by sinusoidal endothelial cells through very efficient receptor-mediated endocytosis. Liver sinusoidal endothelial cells constitutively express all molecules necessary for antigen presentation (CD54, CD80, CD86, MHC class I and class II and CD40) and can function as antigen-presenting cells for CD4+ and CD8+ T cells. Thus, these cells probably contribute to hepatic immune surveillance by activation of effector T cells. Antigen-specific T-cell activation is influenced by the local microenvironment. This microenvironment is characterized by the physiological presence of bacterial constituents such as endotoxin and by the local release of immunosuppressive mediators such as interleukin-10, prostaglandin E2 and transforming growth factor-beta. Different hepatic cell populations may contribute in different ways to tolerance induction in the liver. In vitro experiments revealed that naive T cells are activated by resident sinusoidal endothelial cells but do not differentiate into effector T cells. These T cells show a cytokine profile and a functional phenotype that is compatible with the induction of tolerance. Besides sinusoidal endothelial cells, other cell populations of the liver, such as dendritic cells, Kupffer cells and perhaps also hepatocytes, may contribute to tolerance induction by deletion of T cells through induction of apoptosis.